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**14. ABSTRACT**
Aim I. Development of a novel fluorescence imaging technique, for in vivo detection of retinal pigment epithelium lesions following exposure to laser radiation. We acquired a new custom-built multi wavelength scanning laser ophthalmoscope (SLO) for multi spectral in vivo fluorescence imaging of animal retina following laser exposure. The imaging system was optimized for retinal imaging in aged Brown Norway rats. In order to induce laser lesions in the retina in vivo, we integrated the surgical laser system with our ophthalmoscope and we obtained images of the rat retina from animals following laser exposure. Aim II. Demonstrate that preconditioning of retina by using chronic low dose light exposure or transient sub threshold exposure to thermal insult induces a desirable adaptive response that could provide retina protection against subsequent supra-threshold laser exposure. Duplicate experiments suggested an effect of pre-conditioning, in that the heated animals required a higher laser power to show RPE changes by fundus autofluorescence. Subsequently, we found that post-exposure conditioning showed an even more consistent protection. Pre-and post conditioning upregulated Heat Shock Proteins effectively.

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Multi-modal imaging, Protection against laser exposure, Pre-exposure conditioning, Post-exposure conditioning.

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**FINAL REPORT: January 15, 2006 to May 31, 2009**

**Title: A Novel Approach for Sub threshold Detection and Prevention of Laser Injury in Ocular Tissue**

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Institution: University of Texas Medical Branch

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Dr. Hammer is an expert at in vivo imaging of the eye. He has developed equipment and software to allow adaptive optics based images of the posterior pole of the eye. Dr. Hammer will contribute his equipment and software; we have shown him the benefit of its use in human and rodents.

F. Honors: None

G. Key Findings and Results.

**Aim I. Development of a novel fluorescence imaging technique, for *in vivo* detection of retinal pigment epithelium lesions following exposure to laser radiation.**

We acquired a new custom-built multi wavelength scanning laser ophthalmoscope (SLO) for multi spectral *in vivo* fluorescence imaging of animal retina following laser exposure. The imaging system was optimized for retinal imaging in aged Brown Norway rats of at least 12 months of age and used fundus autofluorescence at 488 nm and 514 nm. These images could be subtracted to allow quantitative analysis of parameters such as lipofuscin density, shown in figure 1. In order to induce laser lesions in the retina in vivo, we integrated the surgical laser system with our ophthalmoscope. Subsequently we obtained white light and AF images of the rat retina from several animals and produced sub-threshold laser lesions which were followed in time as shown in figures 2 and 3. Preliminary histopathology did not show histological changes in he retina following this level

of exposure, figure 3E. This was surprising as the sub-threshold lesions are readily shown with fundus autofluorescence. Histopathology showed no disruption of the retinal layers with laser powers at near threshold, indicating that autofluorescence also is able to detect changes in chemical composition which can not be visualized with histopathology using standard H&E stains. We hypothesize that the autofluorescence induces photo-chemical changes in the tissues which may not lead easily detectable histological changes. In order to explore these results further a second instrument was acquired with OCT capabilities. An example of spectral domain OCT of the Brown Norway rat is shown in figure 4. Due to the extensive disruption caused by hurricane IKE experiments to monitor sub-threshold laser tissue interaction with autofluorescence and OCT are pending at this time and those results will be communicated to the program officer in the future.

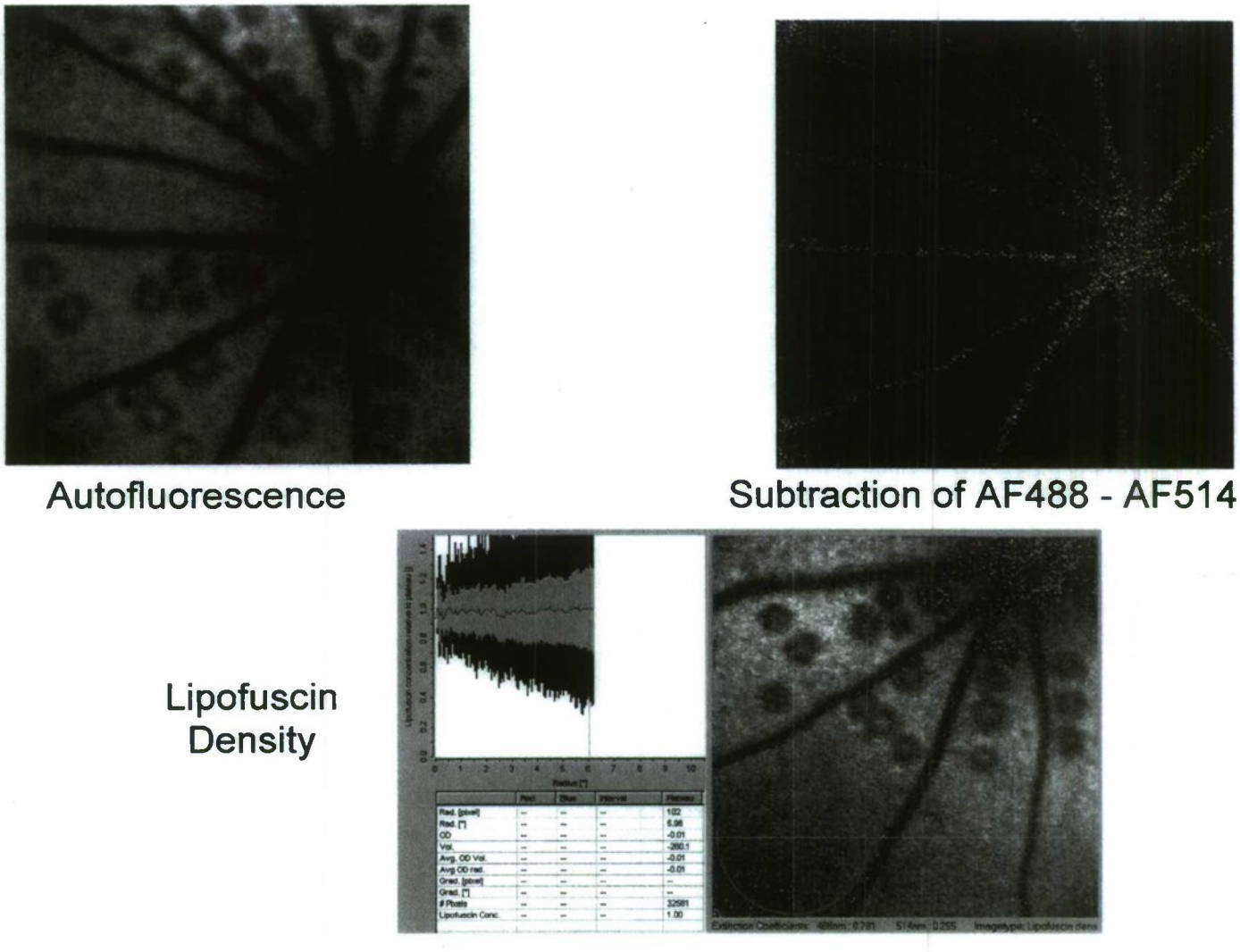



**Figure 1.** Fundus Autofluorescence image at 488 nm of animal following laser exposure (upper left). The changes in autofluorescence are different at 488 and 514 nm, allowing for the appearance of the dark lesions in the subtracted image (upper right). Quantitative analysis of lipofuscin density shows minimal change at this time point (bottom).

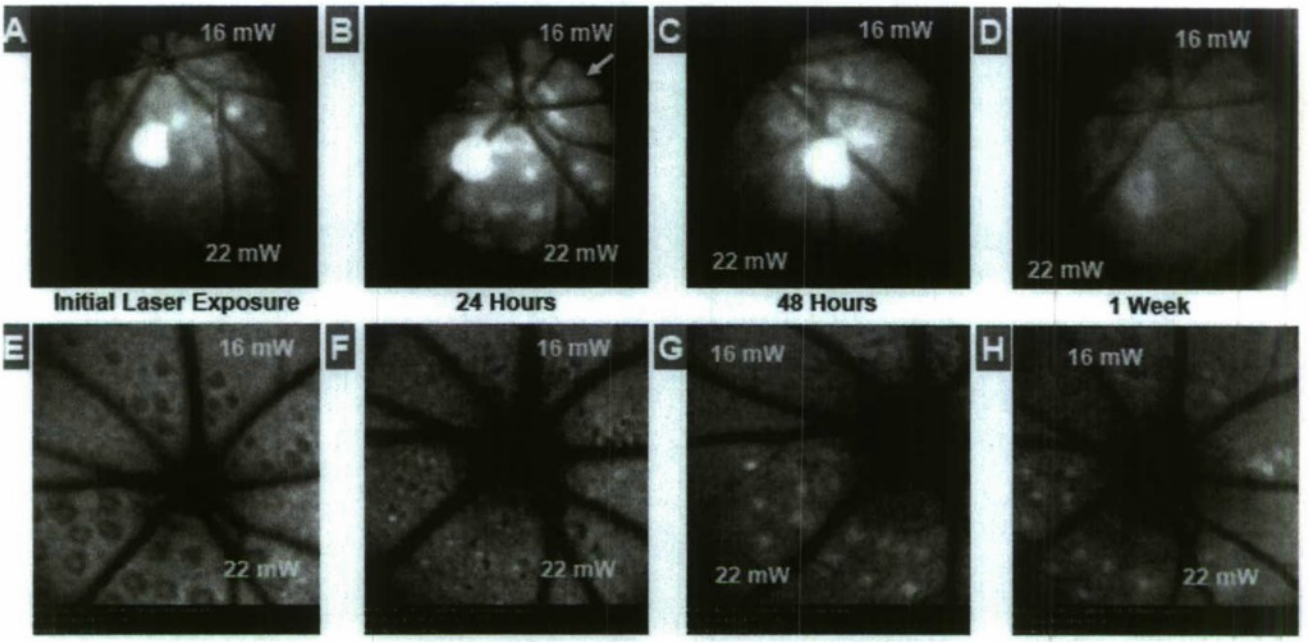
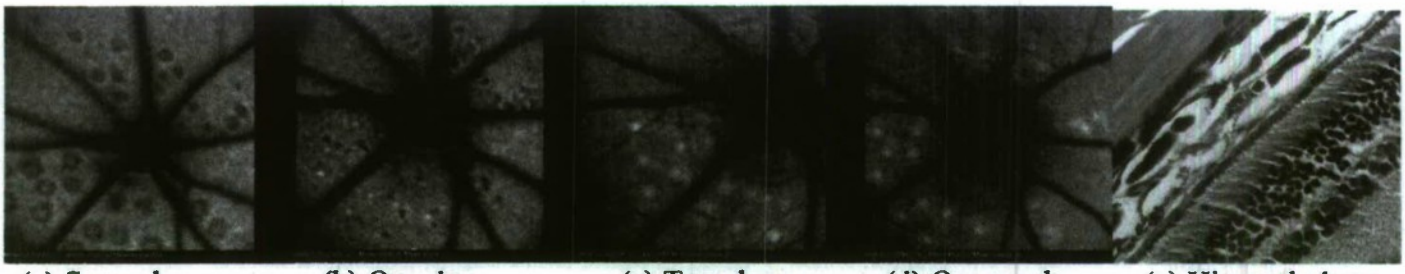



**Figure 2.** A_D white light fundoscopy of near-threshold and supar-threshold laser lesions in a Brown Norway rat. Both 16 mW and 22mW lesions appear as distinct white spots that fade entirely after a period of 48 hours. E-H: corresponding autofluorescence imaging of a Brown Norway rat. (E) AF image immediately after near-threshold and supra-threshold laser exposure (16 and 22 mW). (F) AF image at 24 hours shows the healing process occurring in the 16 mW lesions and the periphery of the 22 mW lesions. (G) AF images at 48 hours exhibit the reappearance of the higher powered lesions as hyperfluorescent spots. (H) In contrast to the white light fundoscopy image. AF imaging 1 week still shows the clearly defined hyperfluorescent spots from 22 mW lesions and amorphous areas corresponding to the 16 mW laser exposure.



(a) Same day (b) One day (c) Two days (d) One week (e) Hisopathology

**Figure 3.** AF image of near-threshold (16 mW, above the disc) and supra-threshold laser (22 mW, below the disc) lesions in a Brown Norway rat. (a) AF image immediately after laser exposures. (b) 24 hour AF image shows the healing process occurring in the 16 mW lesions and the periphery of the 22 mW lesions. (c) 48 hours AF image exhibit the reappearance of the higher powered lesions as hyper-fluorescent spots. (d) 1 week AF image still shows the clearly defined hyperfluorescent spots from the 22 mW lesions and amorphous areas corresponding to the 16 mW laser exposures. (e) Histopathology of sections through the laser lesions does not does not reveal any changes.

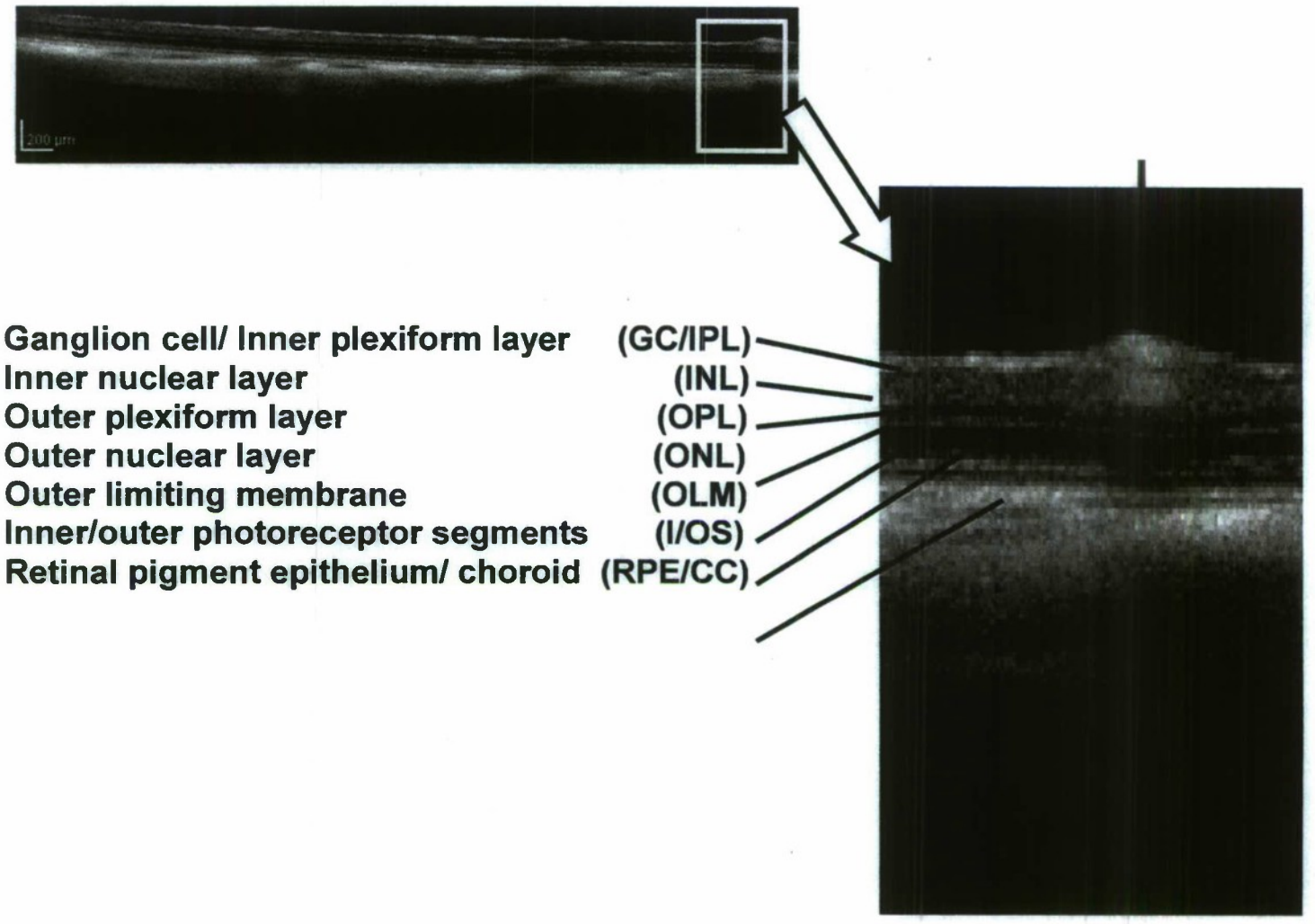



**Figure 4.** Spectral domain OCT imaging of the Brown Norway Rat retina. The various layers in the retina can be easily identified. The prominence near the red arrow is a major blood vessel causing a shadow in the remainder of the image.

**Aim II. Demonstrate that preconditioning of retina by using chronic low dose light exposure or transient sub threshold exposure to thermal insult induces a desirable adaptive response that could provide retina protection against subsequent supra-threshold laser exposure.**

**Description of experiments.** Since we identified the Brown Norway Rat as an excellent animal model several pre-conditioning experiments were carried out. Hyperthermia was induced in the rats to upregulate HSP synthesis by submerging the animals in a water bath at 42°C. The rectal temperature of each animal was monitored to insure that core body temperature remained above 41°C for 20 minutes, figure 6. A Coherent Ultima argon-ion laser operating at 514 nm was used to create photo-thermal damage with an exposure time of 100 ms and a 0.10 mm spot size to photocoagulate the retina of pigmented rats. Upregulation of HSPs was established through quantification of heat shock transcription factors responsible for activation of multiple heat shock proteins (i.e. HSP40/70/90/110) and NFκB was measured to further verify cellular stress response.

**Results.** Duplicate experiments suggested an effect of pre-conditioning, in that the heated animals required a higher laser power to show RPE changes by fundus autofluorescence, figure 5F. This result could not be consistently duplicated initially and further standardization of laser application was required. This was accomplished by using contact lenses during the laser application to avoid aberrations at the cornea interphase. In addition, we developed a color photography system for rodents as shown in figure 2 A-D. It demonstrated that the lesions change with time but that this change can also be easier documented by fundus autofluorescence as shown in figure 5. The data shown in figure 7 indicate a substantial upregulation of HSPs under the conditions used. The experiments were carried out in collaboration with scientists at Brooks City Base who also set up a RPE culture facility which was used for *in vitro* pre-conditioning experiments. They have used the same pre-conditioning parameters for the *in vitro* pre-conditioning experiments that were established at UTMB for the animal model. Lesion Healing in Control Eye vs. Heat Treated Eye was established first using Pre-exposure thermal conditioning. The results can be summarized as follows, see figure 8.

- Initial lesion size in heat treated eye is ~20% less than in the control eye
- Heat Treated eye shows sharp decline in lesion appearance 6 hours after laser exposure
- Final lesion size in both eyes 72 hours post laser are equal despite initial differences
- HSP expression is at a maximum during laser exposure but declines significantly in the following 72 hours

Post-exposure thermal conditioning was done to allow upregulation of HSP closer to the actual laser exposure. Control subjects retain characteristics of heightened retinal damage (i.e. circular shape and areas of high intensity in FAF imaging. Conditioned subjects exhibit signs of decreased retinal damage (i.e. diffuse lesion areas at relatively low intensity values), see figure 9.

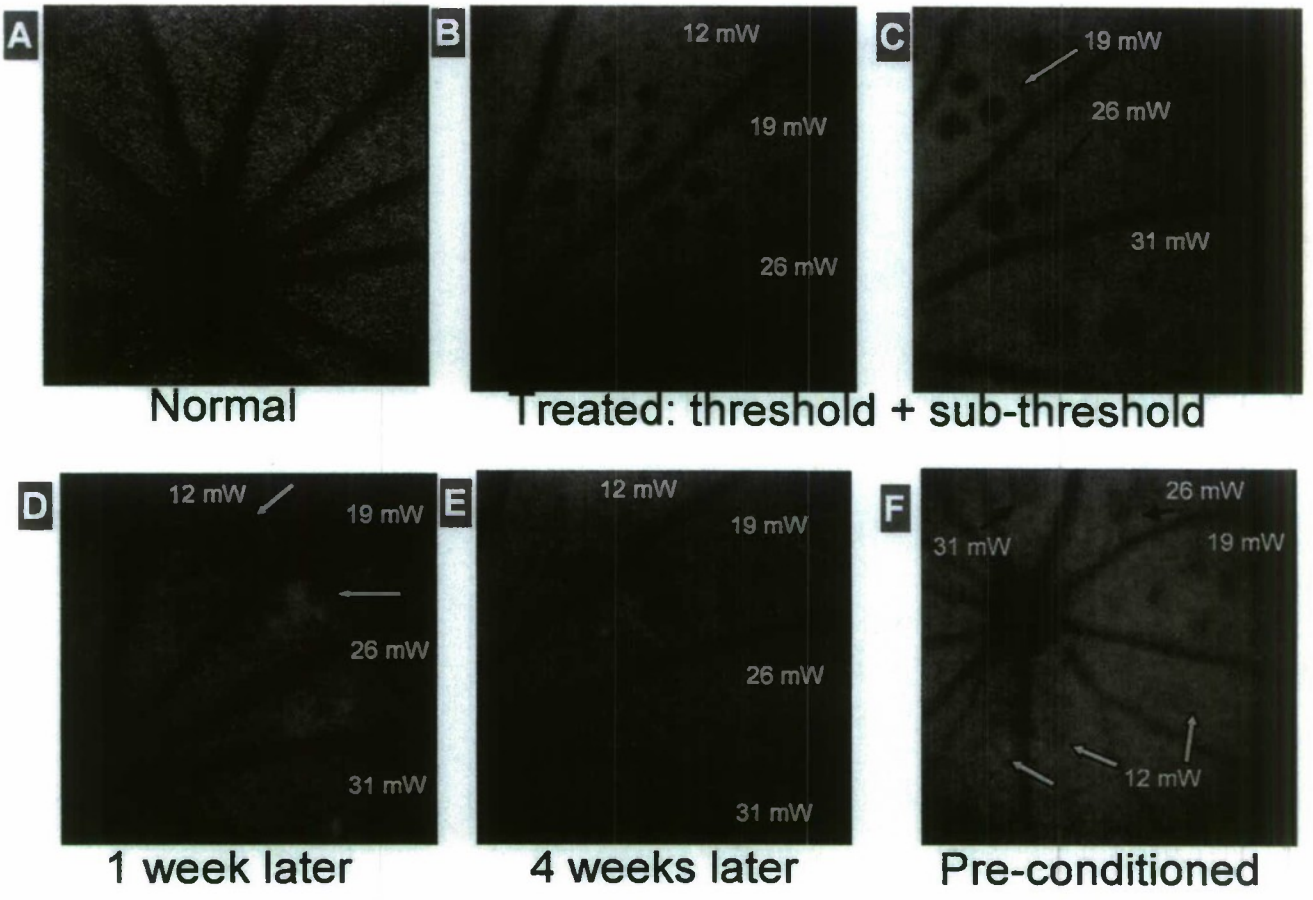



**Figure 5.** **A.** Normal autofluorescence image of Brown Norway rat. **B + C.** AF image immediately after supra-threshold laser (26 or 31 mW, black arrows) and sub-threshold laser (12 or 19 mW, blue arrows). AF images 1 week **(D)** 4 weeks **(E)** and thermally pre-conditioned with heat **(F)** after laser treatment. It shows that there is a healing process, which is most complete in the area of laser with the lowest power settings (group of 6 spots, 12 mW exposure). Most importantly, sub-threshold laser damage was not visible in a heat pre-conditioned animal.

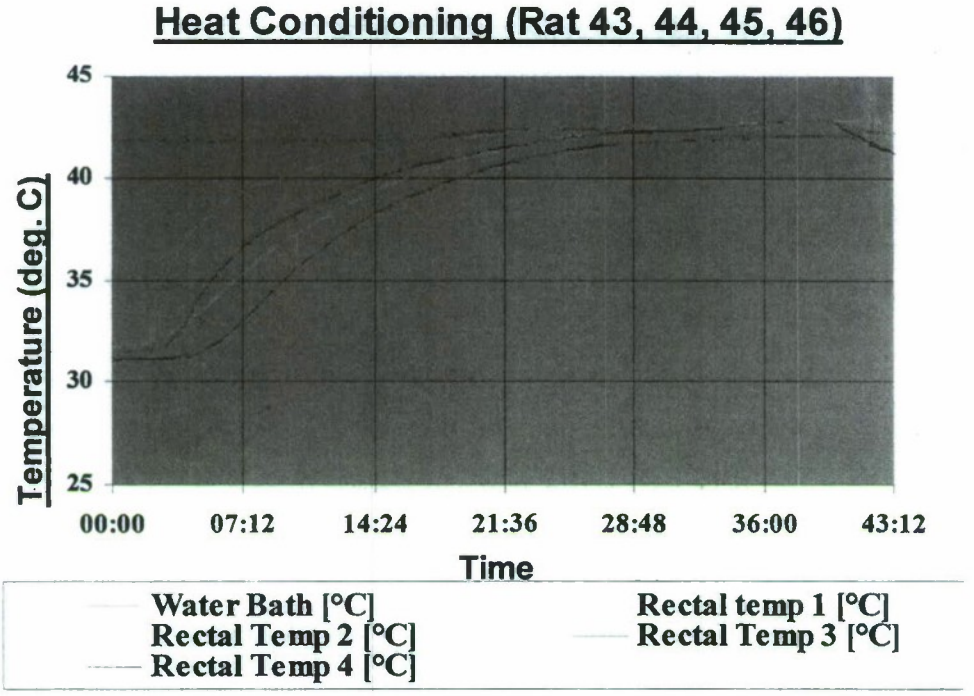
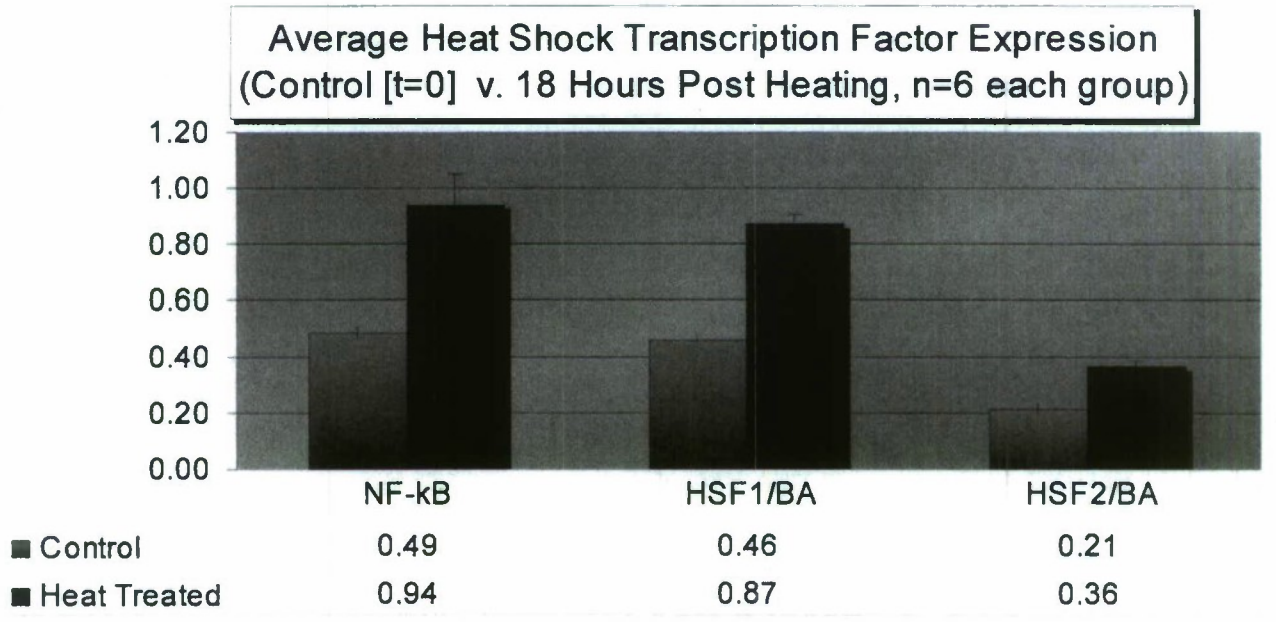
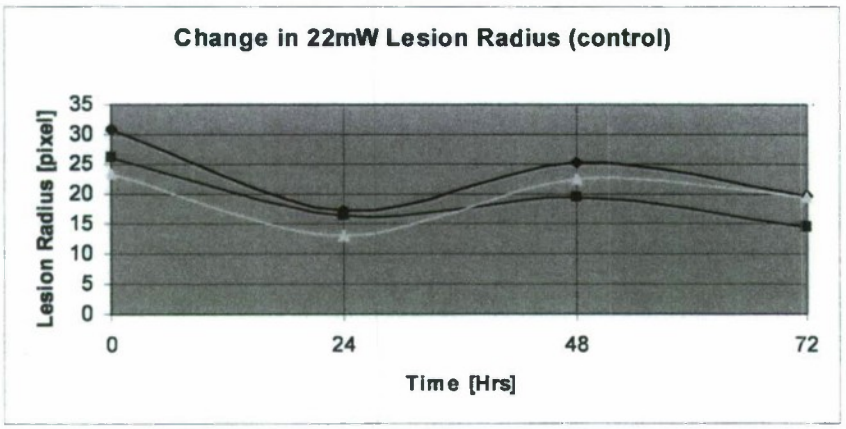



**Figure 6.** Example of heat conditioning of 4 animals using a rectal probe to measure body temperature.




Upregulation of NF-κB and heat shock transcription factors 1 &2.

**Figure 7.** Western Blot Analysis of Stress Response and HSP Upregulation



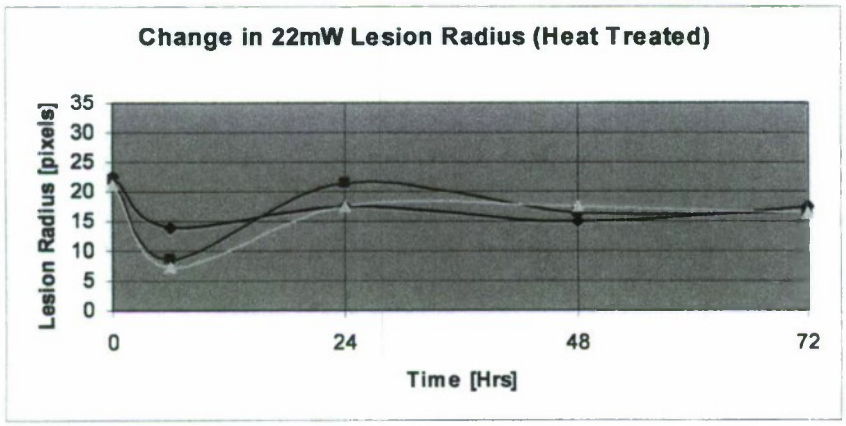




**Figure 8.** Changes in lesion size following laser exposure in Brown Norway Rats, N=6. Thermal conditioning was performed 18 hours prior to laser exposure. Initially the lesions are smaller in the pre-conditioned group of animals. In addition, there is a sharp decline in the lesion of the pre-conditioned animals; however this difference disappears in 72 hours.

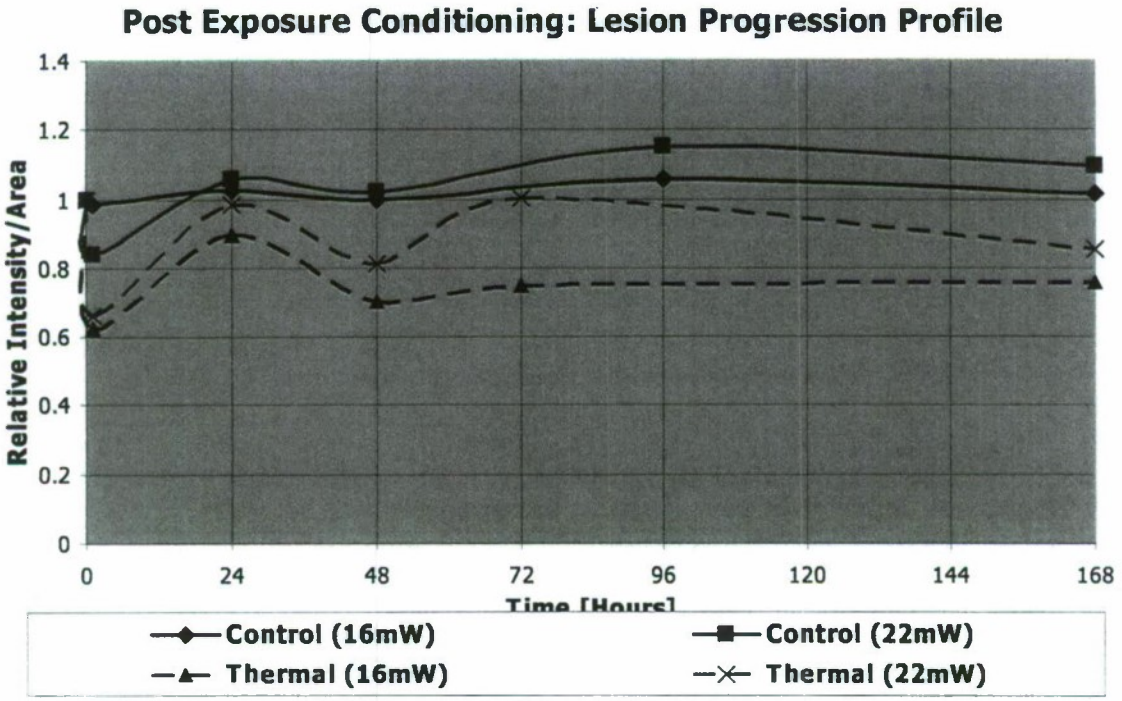



**Figure 9.** Lesion progression at 16 and 22 mW, n=6 animals, 5 lesions evaluated per animal per laser power. Thermal conditioning procedure performed in < 1 hr immediately after laser exposure.

**Conclusions:**

- **Established a new model that enables effective utilization of promising molecular imaging techniques including Fundus autofluorescence (AF).**
- **Demonstrated that AF is a highly sensitive tool for the detection of sub-threshold laser-induced retinal injury.**
- **Demonstrated that AF can be used as an effective non-invasive tool to revaluate MPEL for laser safety and monitor the healing response of retina following laser-induced injury.**
- **Demonstrated that thermal pre-conditioning can be used to improve protection in rat retina against laser exposure**
- **Discovered a novel thermal conditioning approach that can be used to minimize laser-induced injury in the retina following laser exposure**
- **Developed the imaging parameters for multi-modal image-based platform (I.E. AF, IR and OCT) that can be used to evaluate the retinal response to laser-induced injury in small animal models.**
- **Preliminary studies using conventional H & E staining techniques failed to reveal any histological changes in the retina of the animals that received sub-threshold laser treatment and in which laser-induced changes in the autofluorescence features of retina were observed in vivo following laser treatment.**